

ATAD3A confers malignant potential in head and neck cancer via its ATP-binding ability

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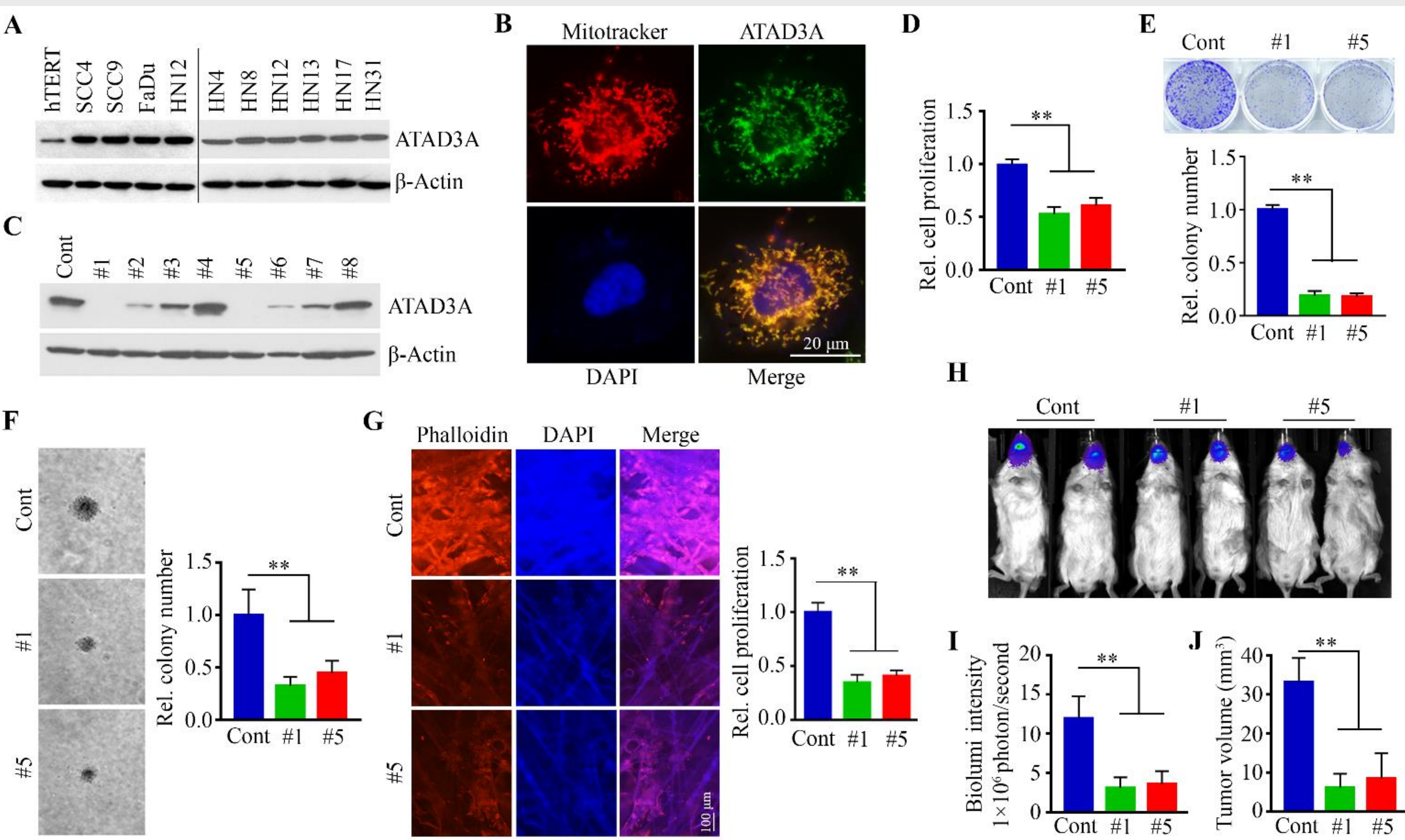
BACKGROUND: There has been a little improvement in the treatment outcomes of head and neck squamous cell carcinoma (HNSCC) in decades, and a better understanding of the molecular biology of HNSCC is urgently needed to facilitate the design of anti-HNSCC therapies. The mitochondrial ATPase family AAA-domain containing protein 3A (ATAD3A) has been demonstrated as an oncogene in breast and lung cancer. However, nothing sheds lights on its role in HNSCC development and progression.

Methods: ATAD3A knockdown and knockout cells were generated using lentiviral-mediated shRNAs and a CRISPR-Cas9. Cancer cell growth was assessed by MTT, colony formation, soft agar, and 3D culture. Tumor development was determined by bioluminescent luciferase signal, tumor size, and weight. The molecular changes under different conditions were examined by Western blotting and immunofluorescence.

Summary of Key Findings

- 1) Loss of ATAD3A expression suppresses HNSCC cell growth and elicits tumor regression in orthotopic tumor-bearing mice, whereas gain of ATAD3A expression produces the opposite effects.
- 2) The tumor suppression induced by the overexpression of Walker A dead mutant of ATAD3A produces a potent dominant-negative effect due to defective ATP-binding.

CENTRAL CONCLUSION: ATAD3A drives HNSCC tumorigenesis and abrogating its ATP-bind ability represents a promising anti-HNSCC regimen.



Results

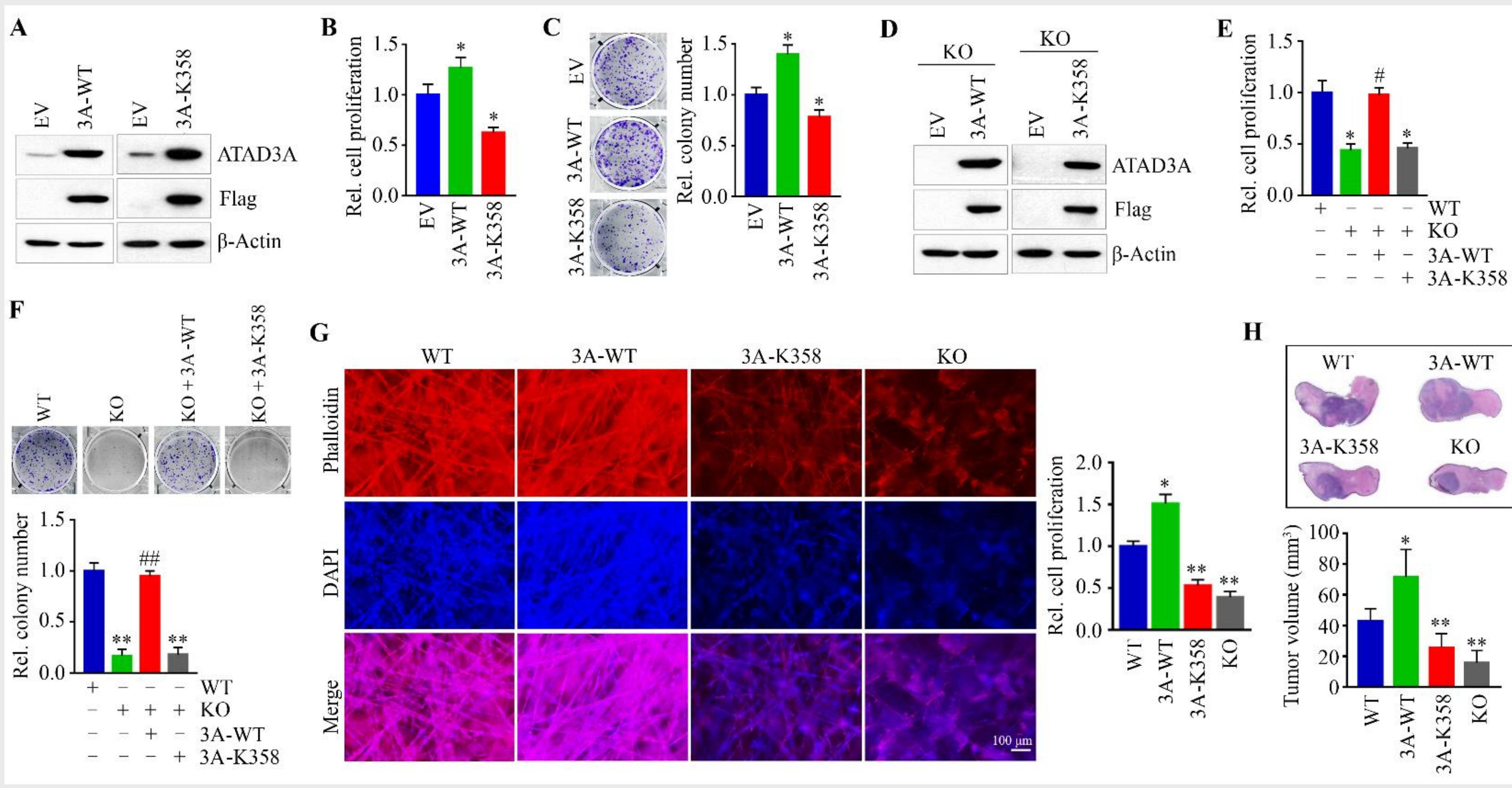


Figure 2. Knockout of ATAD3A inhibits tumor development in HNSCC. (A) ATAD3A protein levels in various HNSCC cell lines. (B) The subcellular localization of ATAD3A in HN12 cells determined by confocal microscopy. (C) The ATAD3A knockout clones derived from the sgATAD3A-targeting HN12 cells screened by Western blotting. (D) The effect of ATAD3A knockout (#1 and #5) on cell proliferation on day 3. (E) The effect of ATAD3A knockout on cell colony formation within 3 weeks. (F) The effect of ATAD3A knockout on soft agar cell growth on day 14. (G) The effect of ATAD3A knockout on HN12 cell growth on day 14 in 3D SeedEZ scaffold. (H-I) The effect of ATAD3A knockout on HN12-derived tongue tumor growth in NSG mice. Tumor progression was monitored on day 15 by examining bioluminescence in Xenogen IVIS-200 In Vivo Imaging System. Representative bioluminescence images and quantitative data were respectively shown in (H) and (I), and tumor volume was shown in (J). hTERT: Human telomerase-immortalized tonsillar keratinocytes hTERT HAK Clone 41 ***p*<0.01.